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Original Article

Transrectal Doppler sonography of uterine blood flow during the first two weeks after parturition in Simmenthal heifers

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Transrectal Doppler sonography was used to evaluate uterine blood flow during the first two weeks after parturition in six primiparous Simmenthal cows. The uterine blood flow was evaluated on the day of parturition (Day 0), once daily from Days 1 to 8 and then every other day until Day 14. Blood flow was quantified by determining the diameter (D), the time-averaged maximum velocity (TAMV), the pulsatility index (PI) and the blood flow volume (BFV) of the uterine arteries ipsilateral and contralateral to the formerly pregnant uterine horn. During the first four days after calving D, TAMV and BFV declined (ipsilateral: TAMV 70 %, BFV 87 %, contralateral: D 47 %, BFV 84 %; $p < 0.05$), while PI increased (ipsilateral 158 %, contralateral 100 %; $p < 0.05$) distinctly. Between Days 4 and 14 only the ipsilateral D (12 %) and the BFV of both arteries (ipsilateral 5 %, contralateral 8 %) decreased ($p < 0.05$). Blood flow variables were very strongly correlated with each other ($r > \pm 0.75$, $p < 0.05$), with negative correlations with PI and positive correlations with all other investigated factors. Overall, this study revealed characteristic changes in uterine perfusion during the first two weeks after parturition in cows that were pronounced during the first four days postpartum.

Keywords: involution, puerperal, uterus

Introduction

In dairy cows, the puerperal period is a critical phase in the reproductive cycle, during which uterine involution occurs [7]. Abnormal uterine involution results in a marked decrease in fertility [5]; therefore, a thorough understanding of the processes involved in uterine involution is important for successful management of dairy cows during early lactation.

Until recently, methods for non-invasive investigation of uterine involution of cows were limited. Transrectal palpation of the uterus is probably the most commonly used method, but this is a subjective evaluation [2,19] and, similar to B-mode ultrasonography [1,2,12,15,16,19], cannot be used to evaluate the entire uterus in the early postpartum period [1,12,19].

Doppler sonographic evaluation of uterine blood flow has been used for several years to identify postpartum uterine involution in women. Studies conducted using this technique showed an association between uterine blood flow and delayed uterine involution [11,13,18,22]. In cows, Doppler sonography has been used to assess uterine blood flow during the estrous cycle [4] and pregnancy [3,10,20], as well as in the postpartum period [14], although the intervals between examinations were relatively long in the latter study. Because clinical studies have shown that uterine changes in normal cows occur rapidly during the first few days postpartum, our study was conducted to assess the physiological uterine perfusion at frequent intervals *via* Doppler sonography during the early puerperal period. This technique is non-invasive and could serve as an objective tool for the assessment of uterine involution provided that reproducible measurements are obtained.

Materials and Methods

Investigations were carried out in six, two-year-old, primiparous, Simmenthal cows. All cows were kept in straw-bedded tie stalls, fed hay and grain and milked twice a day. Color Doppler sonographic studies of the uterine arteries started three hours after parturition (= Day 0), and were followed by daily examinations until Day 8. Thereafter, blood flow measurements were carried out

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every other day until Day 14. General clinical examinations were conducted every day during this period.

Both uterine arteries were identified using transrectal color Doppler sonography as described previously [3]. The uterine artery ipsilateral to the formerly pregnant horn is referred to as the ipsilateral artery, while the uterine artery contralateral to the formerly pregnant horn is referred to as the contralateral artery. Examinations lasted an average of 30 min for each cow, and Doppler sonography was carried out using a SSH 140 A ultrasound machine (Toshiba, Japan). Because blood flow velocities were high until Days 4 to 8 after parturition, Doppler sonographic measurements were conducted using a 3.75 MHz sector transducer with a 400 Hz high-pass filter in continuous mode. From Day 10, the blood flow velocities were slower and could be measured in pulsed-wave mode using a 7.0 MHz microconvex transducer with a 50 Hz high-pass filter. Blood flow waveforms were obtained at an interrogation angle of 20 to 60 degrees between the Doppler ultrasound beam and flow direction. All observations were videotaped. After each Doppler sonographic examination, B-mode sonography was used to determine the diameters of both uterine arteries. For further evaluations, the means of three measurements of vessel diameter made during an examination were used.

Doppler calculations were completed off-line using two similar consecutive flow velocity waveforms with maximum frequency shifts. The uterine blood flow was assessed using the diameter of the uterine artery (D), the time-averaged maximum blood flow velocity (TAMV), the blood flow volume (BFV) and the pulsatility index (PI). All variables were calculated according to Bollwein *et al.* [3].

Statistical analyses were carried out using the Statistical

Analysis System (SAS Institute, USA). The Shapiro-Wilk test was used to test for normality of the distribution of all blood flow variables. Because the distributions of all variables (D, PI, TAMV and BFV) were not normal, the non-parametric Wilcoxon's signed rank test (PROC UNIVARIATE; SAS Institute) was used to analyze differences in blood flow values between the ipsilateral and contralateral arteries, as well as changes in blood flow variables between days within animals. Friedman two-way ANOVA was used to determine the effects of the day postpartum on repeated measurements (PROC FREQ; SAS Institute). Correlations between blood flow variables were tested by Spearman's rank correlation (PROC CORR; SAS Institute), with correlations between 0.50 and 0.75 being considered strong and those greater than 0.75 very strong. Correlation coefficients were calculated using the means of all days of examination to analyze the effects of time postpartum. To examine correlations between various blood flow variables immediately after calving on Day 0 and 1, single values separated by the side of the arteries were used. Values of $p < 0.05$ were considered significant.

Results

Clinical findings

All six cows were clinically healthy during the examination period. The duration of pregnancy varied from 276 to 290 days (mean, 286.5 days). In all cows, parturition was unassisted, expulsion of the placenta was completed within 12 hours of calving and on Day 14 postpartum the uterus could be retracted into the pelvic cavity during transrectal palpation. The reported percent changes in the following blood flow variables were always related to the value on Day 0.

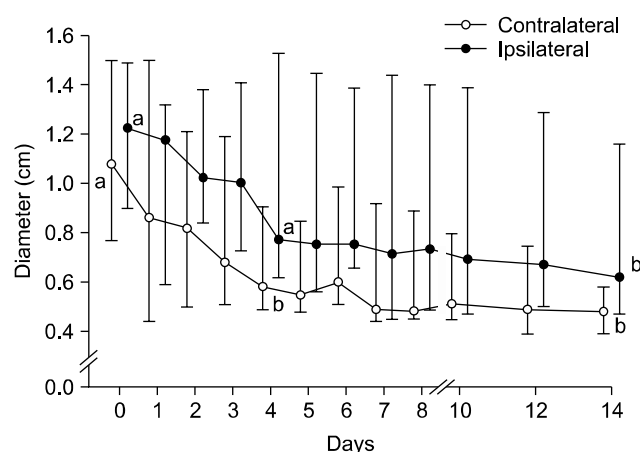


Fig. 1. Medians and ranges of the diameters of the ipsilateral and contralateral uterine arteries between Day 0 (immediately after parturition) and Day 14 postpartum in six healthy cows. Within an artery, medians with different letters on Day 0, 4 and 14 are different ($p < 0.05$).

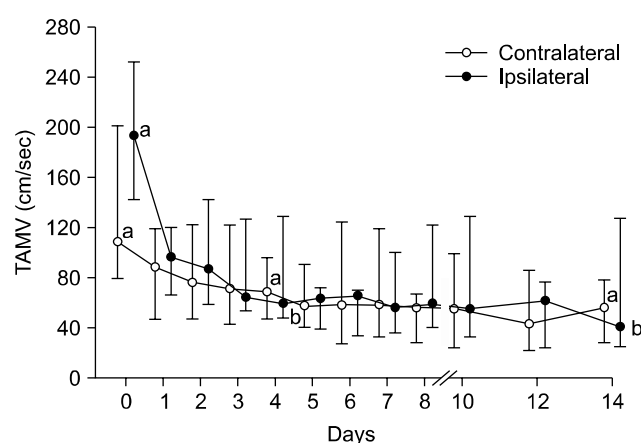


Fig. 2. Medians and ranges of the time-averaged maximum velocities (TAMV) of the ipsilateral and contralateral uterine arteries between Day 0 (immediately after parturition) and Day 14 postpartum in six healthy cows. Within an artery, medians with different letters on Day 0, 4 and 14 are different ($p < 0.05$).

Diameter of the uterine arteries

The ipsilateral uterine artery was larger ($p < 0.0001$) than the contralateral artery (Fig. 1). From Day 0 to 4, the diameters of the ipsilateral and contralateral arteries decreased by 37% and 47%, respectively, but only the decrease in the contralateral artery was significant ($p < 0.05$). From Day 4 to 14, the diameter of the ipsilateral uterine artery decreased significantly (12%) ($p < 0.05$), while the diameter of the contralateral vessel (9%) decreased, but not significantly ($p > 0.05$) when compared to the value on Day 4.

Time-averaged maximum velocity

There was no difference in the TAMV of the ipsilateral and contralateral arteries ($p < 0.05$) (Fig. 2). By Day 4, the TAMV in the ipsilateral uterine artery had decreased ($p < 0.05$) relative to Day 0. Specifically, a decrease of 50% occurred between Day 0 and 1 and a further decrease of 20% (of the Day-0 value) occurred between Day 1 and 4. During the remaining examination period, the TAMV of the ipsilateral artery did not change ($p > 0.05$). The TAMV

of the contralateral artery remained unchanged ($p > 0.05$) throughout the study period.

Blood flow volume

The BFV was higher in the ipsilateral artery than in the contralateral artery ($p < 0.0001$), and decreased in both the ipsilateral and contralateral uterine arteries during the first 4 days after parturition ($p < 0.05$). In the ipsilateral artery, the BFV decreased 50% between Day 0 and 1 and another 37% (of the Day-0 value) between Day 1 and 4. In the contralateral uterine artery, the BFV fell 36% between Day 0 and 1 and another 48% (of the Day-0 value) between Day 1 and 4. After Day 4, the BFV decreased another 5% ($p < 0.05$) in the ipsilateral artery and another 8% ($p < 0.05$) in the contralateral artery until Day 14 (Fig. 3).

Pulsatility index

The PI was dependent ($p < 0.05$) on the cow but not the location ($p > 0.05$) (ipsilateral versus contralateral). The PI increased in both arteries between Day 0 and Day 4 (ipsilateral, 158%; contralateral, 100%; both $p < 0.05$), and showed only mild changes ($p > 0.05$) after Day 4 (Fig. 3).

Correlation between blood flow variables

The PI was very strongly and negatively correlated with the other three blood flow variables, and there were very strong positive correlations between D and TAMV, D and BFV, and TAMV and BFV in both uterine arteries (all $r > 0.75$, all $p < 0.05$; Table 1).

On Days 0 and 1, there were strong correlations between TAMV and BFV, TAMV and PI and BFV and PI of the ipsilateral artery and between D and BFV and TAMV and PI of the contralateral uterine artery (Table 2).

Discussion

There were characteristic changes in all blood flow variables during the first two weeks postpartum. The BFV showed the largest changes in the first four days postpartum. The distinct alterations in this time period were most likely attributable to the tremendous decrease in the size and weight of the uterus. This may be explained, at least in part, by myometrial contractions, which occur as

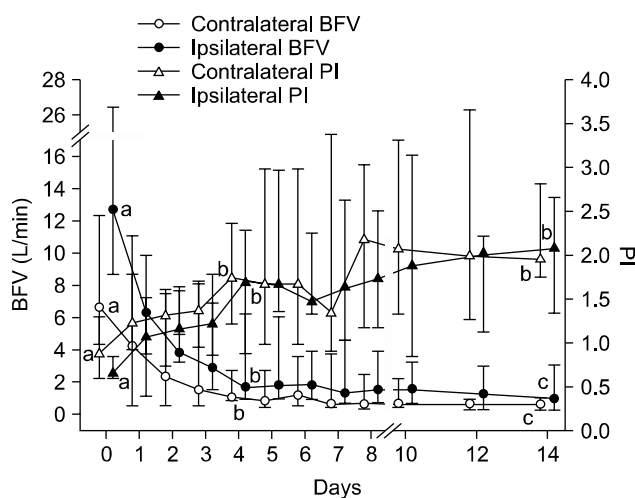


Fig. 3. Medians and ranges of the blood flow volume (BFV) and the pulsatility index (PI) in the ipsilateral and contralateral uterine arteries between Day 0 (immediately after parturition) and Day 14 postpartum in six healthy cows. Within an artery, medians with different letters on Day 0, 4 and 14 are different ($p < 0.05$).

Table 1. Spearman's rank correlation coefficients for the relationships between the medians of diameter (D), time-averaged maximum velocity (TAMV), blood flow volume (BFV) and pulsatility index (PI) of the ipsilateral and contralateral uterine arteries

Uterine artery	D / TAMV	D / BFV	D / PI	TAMV / BFV	TAMV / PI	BFV / PI
Ipsilateral	0.85*	0.96*	-0.92*	0.90*	-0.84*	-0.92*
Contralateral	0.85*	0.96*	-0.96*	0.82*	-0.76*	-0.96*

* $p < 0.05$. In each variable, all 12 measured values were used for calculation.

Table 2. Spearman's rank correlation coefficients for the relationships between the diameter (D), time-averaged maximum velocity (TAMV), blood flow volume (BFV) and pulsatility index (PI) of the ipsilateral and contralateral uterine arteries on Days 0 and 1

Uterine artery	D / TAMV	D / BFV	D / PI	TAMV / BFV	TAMV / PI	BFV / PI
Ipsilateral	0.4	0.68*	−0.55	0.87*	−0.81*	−0.83*
Contralateral	0.05	0.83*	−0.28	0.44	−0.78*	−0.61*

* $p < 0.05$.

strong, rhythmic, peristaltic waves during the first three days after calving. These contractions result in reduction of muscle fiber length from about 750 μ to 200 μ by Day 3 post partum [23]. Expulsion of most of the lochia in the first few days postpartum is another important factor in the decrease in size and weight of the uterus [8,23]. Studies have shown that the weight of the uterus decreases by about 50 [8] to 66% [17] in the first five days postpartum. In comparison, the BFV in the uterine arteries of the cows in the present study decreased over 80% in the first four days postpartum. From Day 5 to Day 14 postpartum, the weight of the uterus decreases an additional 30% [8], while the BFV decreases an additional 9%. Invasive methods using a blood flow transducer showed similar reductions in BFV in the first few days postpartum [6,9]. However, in those studies, BFV was only assessed in the uterine artery ipsilateral to the previously pregnant horn, presumably because of the invasiveness of the procedure, and only during the first four to five days postpartum. Guilbault *et al.* [9] reported that the BFV decreased by 84% in the first four days postpartum in five Jersey and Holstein cows. Ford *et al.* [6] reported a linear decrease in BFV in three Hereford cows in the first five days after calving, although the magnitude of the decrease was not described. In another study using transrectal color Doppler sonography, the BFV in the uterine arteries of pluriparous Holstein-Friesian cows decreased by more than 70% between Day 1 and Day 7 postpartum [14]. However, unlike the present study, more precise data about the decrease in BFV in this time period was not possible because of the large interval between sonographic evaluations. The observation that the BFV decreased by 15% between Day 7 and Day 14 was in general agreement with the results of our study [14].

The BFV was calculated using a formula that incorporated the D and the TAMV. There were strong correlations between D and BFV and between TAMV and BFV throughout the study. The correlation between D and BFV (0.68) was weaker than that between TAMV and BFV (0.87) in the ipsilateral uterine artery on Days 0 and 1, indicating that the marked decrease in blood flow in this time period was primarily due to a reduction in blood flow velocity and to a lesser extent to a decrease in the diameter of the artery.

The PI only increased in the first four days postpartum and did not change significantly thereafter. A similar increase also occurred in a previous study, but in contrast to the present investigation, it was significant at Day 7, and continued until Day 14 postpartum [14]. There were strong correlations between PI and BFV throughout the study period, but these correlations were distinctly weaker for Days 0 and 1 combined, especially in the contralateral uterine artery, and the BFV decreased exponentially while the PI increased in a linear fashion. These findings indicate that, in the first few days postpartum, BFV cannot be determined reliably using PI. This is in contrast to human medicine, where PI is the main variable used for quantification of BFV in the uterine artery in women [13,21,22].

In conclusion, the results of this study show that there are characteristic changes in uterine perfusion during the early postpartum period in heifers, which can be evaluated by transrectal color Doppler sonography. Alterations in uterine blood flow are most pronounced during the first four days after calving. Because of the good agreement between flow characteristics and clinical findings, Doppler sonography appears to be an ideal non-invasive technique for the objective evaluation of uterine involution in cattle. However, these findings should be validated in a larger study. It is expected that Doppler sonography will also prove useful for monitoring the outcome of therapeutic measures taken to improve uterine involution in cows with postpartum disease.

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